

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07B 57/00, C07C 213/10 C07C 231/20, C07D 417/04	A1	(11) International Publication Number: WO 93/09075
		(43) International Publication Date: 13 May 1993 (13.05.93)

(21) International Application Number: PCT/SE92/00751

(22) International Filing Date: 30 October 1992 (30.10.92)

(30) Priority data:
9103234-2 4 November 1991 (04.11.91) SE(71)(72) Applicants and Inventors: FISCHER, Lutz [DE/DE];
Ludwigstrasse 18, D-3300 Braunschweig (DE). MÜLL-
ER, Ralf [DE/DE]; Gorch-Fock Strasse 25, D-2359
Henstedt-Ulzburg 3 (DE). MOSBACH, Klaus [SE/SE];
Lackalånga 31, PI 5548, S-244 94 Furulund (SE). EK-
BERG, Björn [SE/SE]; Vinstrupsgratan 12, S-222 22
Lund (SE).(74) Agent: AWAPATENT AB; Box 5117, S-200 71 Malmö
(SE).(81) Designated States: JP, US, European patent (AT, BE, CH,
DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published

With international search report.
In English translation (filed in Swedish).(54) Title: METHOD FOR SEPARATING ENANTIOMERS OF ARYLOXIPROSPANOLAMINE DERIVATIVES, AND
CHIRAL SOLID-PHASE CHROMATOGRAPHY MATERIAL FOR USE IN THE METHOD

(57) Abstract

A method for separating enantiomers of derivatives of aryloxipropanolamines is disclosed. In the method, the derivative is contacted with a chiral solid-phase chromatography material containing molecular imprints of an optically pure enantiomer of the derivative to be separated. A chiral solid-phase chromatography material for use in the method is also disclosed. This material consists of a polymer prepared by polymerisation of a monomer in the presence of a cross-linking agent and of an optically pure enantiomer of the derivative to be separated, a molecular imprint of the optically pure enantiomer being formed in the polymer by non-covalent interactions between the monomer and the optically pure enantiomer. Moreover, there is disclosed the use of the molecular imprinting method for preparing a chiral solid-phase chromatography material for use in the method.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SU	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

METHOD FOR SEPARATING ENANTIOMERS OF ARYLOXIPROPANOLAMINE
DERIVATIVES, AND CHIRAL SOLID-PHASE CHROMATOGRAPHY
MATERIAL FOR USE IN THE METHOD

5 The present invention relates to a method for
separating enantiomers of derivatives of aryloxipropanol-
amines and a chiral solid-phase chromatography material
for use in the method. The invention also relates to the
use of the molecular imprinting method for preparing the
10 chiral solid-phase chromatography material.

β -adrenergic blocking compounds (or β -blockers) are
important pharmaceutical preparations which are used in
the treatment of hypertension, arrhythmia and angina
pectoris. There is a great need of using optically pure
15 enantiomers since the stereoisomers express a varying
pharmacological activity, and in some cases they can also
be used against various symptoms (1). Consequently,
intensive research is in progress for preparing optically
pure β -blockers, e.g. by using an asymmetric synthesis
20 (2) including biocatalysts (3), a fractional crystallisa-
tion (4), as well as indirect (5) or direct (6) chromato-
graphic separation of the enantiomorphs.

According to the present invention, a method is
provided for separating enantiomers of derivatives of
25 aryloxipropanolamines by means of a chiral solid-phase
chromatography material (Chiral Stationary Phase = CSP),
which is prepared by the so-called molecular imprinting
method (7). The molecular imprinting method used is based
on non-covalent complementary interactions between the
30 non-derivatised print molecule and polymerisable monomers.

More precisely, a method is provided for separating
enantiomers of derivatives of aryloxipropanolamines, in
which the derivatives are contacted with a chiral solid-
phase chromatography material containing molecular imprints
35 of an optically pure enantiomer of the derivatives to be
separated.

Moreover, a chiral solid-phase chromatography material is provided to be used in the separation of enantiomers of derivatives of aryloxiopropanolamines, which material consists of a polymer prepared by polymerisation
5 of a monomer in the presence of a cross-linking agent and of an optically pure enantiomer of the derivatives to be separated, a molecular imprint of the optically pure enantiomer being formed in the polymer by non-covalent interactions between the monomer and the optically pure
10 enantiomer.

According to the invention, there is also provided the use of the molecular imprinting method for preparing a chiral solid-phase chromatography material to be used in the separation of enantiomers of derivatives of aryloxi-
15 propanolamines.

Suitable monomers for preparing the chiral solid-phase chromatography material are monomers with functional groups, such as carboxyl-functional monomers. Preferred monomers are methacrylic acid [MAA, $\text{H}_2\text{C}=\text{C}(\text{CH}_3)-\text{COOH}$] or
20 itaconic acid [ITA, $\text{H}_2\text{C}=\text{C}(\text{COOH})-(\text{CH}_2)-\text{COOH}$]. These two monomers can, by their functional groups, form non-covalent bonds in organic solvents to the print molecule. Itaconic acid has previously been used in polymer chemistry (8), but it has now surprisingly been found that this acid is highly
25 suited for use as monomer in the preparation of molecular imprints.

The monomers are polymerised in the presence of a cross-linking agent, which results in a three-dimensional network being formed. One cross-linking agent is ethylene
30 glycol dimethacrylate.

Furthermore, the monomers are polymerised in the presence of a so-called print molecule, i.e. in this case an optically pure enantiomer of the derivative to be separated. During the polymerisation, non-covalent com-
35plementary interactions arise between the non-derivatised print molecule and the polymerisable monomers. After the polymerisation, the print molecule is removed from the

three-dimensional network by extraction with a suitable solvent. As a result, individual sites with complementary points of bonding will probably remain within the polymer.

As aryloxiopropanolamine derivatives that can be
5 separated by the method according to the invention, mention can be made of timolol, propranolol, metoprolol and atenolol (formulae, see Fig. 1).

The resolution of racemic mixtures of important non-derivatised pharmaceutical preparations, such as
10 β -blockers, by using the molecular imprinting method brings great advantages, such as extremely pure preparations and a simple method without any complicated purification steps. With the materials tested according to the Examples, the separation properties were maintain-
15 ed for as long a period as 8 months and with more than 50 injections.

Contrary to previous methods, the method according to the invention offers a high degree of freedom, since it allows the preparation of specific materials with
20 predictable selectivities as desired. By using the described method, it should be possible also on a technical scale to remove contaminating small amounts of an undesired enantiomer.

The invention will now be described in more detail
25 by means of the following Examples and the accompanying Figures.

The expressions and abbreviations used in the Examples have the following meanings:

- R_S = resolution
30 $k'_R = (t_R - t_0)/t_0$
 $k'_S = (t_S - t_0)/t_0$
 $\alpha = K'_S/k'_R$
 k'_R = the capacity factor for the (R)-(+)-enantiomer
 k'_S = the capacity factor for the (S)-(-)-enantiomer
35 k'_{rac} = the capacity factor for the racemate

t_0 = the retention period for non-retained, dissolved substances, the retention period being determined by injecting acetone

R_S determined graphically (7)

5 Fig. 1 illustrates structures of various tested β -blockers. The (S)-(-)-configuration of timolol was used as print molecule for the preparation of the chiral stationary phase (CSP).

Fig. 2 shows a diagram of chromatographic resolution
10 of timolol on polymers containing (A) methacrylic acid and (B) itaconic acid.

Example 1

Preparation of a polymer selective for (S)-(-)-timolol:

632.8 g (2 mmol) (S)-(-)-timolol is resolved in a 50
15 ml test tube in a mixture of 16 ml tetrahydrofuran, 1561.2 mg (12 mmol) itaconic acid, 12.48 ml (60 mmol) ethylene glycol dimethacrylate and 180 mg (1.1 mmol) 2,2'-azobis(2-methylpropionitrile). The solution is cooled in an ice bath, and nitrogen gas is caused to flow through the
20 solution for 20 min. The test tube is sealed. The tube is placed in a freezing chamber (-20°C) and exposed to UV-light at the wave length of 366 nm for 24 h.

The polymer formed is manually ground up in a mortar and then dried in a vacuum-type desiccator for 3 h. The
25 polymer is further ground in a mechanical mortar device (Retsch, Haan, Germany) for 20 min. The material is screened through a 25 μ m screen. The remaining material is ground and screened in two more turns. Small polymer particles from the screened material are removed by a
30 sedimentation process in acetonitrile for 30 min in five turns.

The resulting material is packed in an HPLC steel column (200 x 4.6 mm) in chloroform/acetonitrile (v/v, 3/17) at a pressure of 300 bars.

35 The column is arranged in an HPLC device (LKB, Bromma, Sweden) and washed therein with acetic acid/acetonitrile (v/v, 1/4) at a flow rate of 1 ml/min, for 1 h. Subsequent-

ly, the column is equilibrated in ethanol/tetrahydrofuran/acetic acid (v/v/v, 50/40/10) at a flow rate of 1 ml/min, a pressure of 30 bars and detection at 294 nm.

20 μ g (R,S)-timolol is injected in 20 μ l of the eluant
5 ethanol/tetrahydrofuran/acetic acid (v/v/v, 50/40/10).

$k'_R=1.4$ $k'_S=3.5$ $\alpha=2.5$ $R_S=1.9$

Example 2

Preparation of a polymer selective for (S)(-)-propranolol:

389.01 mg (1.5 mmol) (S)(-)-propranolol is resolved in
10 a 50 ml test tube in a mixture of 6 ml chloroform, 537 mg
(6 mmol) methacrylate, 4.985 ml (24 mmol) ethylene glycol
dimethacrylate and 56 mg (0.34 mmol) 2,2'-azobis(2-methyl-
propionitrile). The solution is cooled in an ice bath, and
15 nitrogen gas is caused to flow through the solution for 20
min. The test tube is sealed. The tube is placed in a cooling
chamber (+4°C) and exposed to UV-light at the wave
length of 366 nm for 24 h.

The polymer formed is ground up manually in a mortar
and then dried in a vacuum-type desiccator for 3 h. The
20 polymer is further ground in a mechanical mortar device
(Retsch, Haan, Germany) for 20 min. The material is screened
through a 25 μ m screen. The remaining material is ground
and screened in two more turns. Small polymer particles
from the screened material are removed by a sedimentation
25 process in acetonitrile for 30 min in five turns.

The resulting material is packed in an HPLC steel
column (200 x 4.6 mm) in chloroform/acetonitrile (v/v,
3/17) at a pressure of 300 bars.

The column is arranged in an HPLC device (LKB, Brom-
30 ma, Sweden) and washed therein with acetic acid/aceto-
nitrile (v/v, 1/9) at a flow rate of 1 ml/min for 1 h.
Subsequently, the column is equilibrated in acetonitrile/
acetic acid (v/v, 93/7) at a flow rate of 1 ml/min, a
pressure of 30 bars and detection at 275 nm.

20 μg (R,S)-propranolol is injected in 20 μl of the eluant acetonitrile/acetic acid (v/v, 93/7).

$k'_R=1.0$ $k'_S=2.8$ $\alpha=2.8$ $R_S=1.4$

Example 3

5 Preparation of a polymer selective for (S)(-)-atenolol:

399.5 mg (1.5 mmol) (S)(-)-atenolol is resolved in a 50 ml test tube in a mixture of 6 ml chloroform, 537 mg (6 mmol) methacrylate, 4.985 ml (24 mmol) ethylene glycol dimethacrylate and 10 mg (0.34 mmol) 2,2'-azobis(2-methyl propionitrile). The solution is cooled in an ice bath, and nitrogen gas is caused to flow through the solution for 20 min. The test tube is sealed. The tube is placed in a cooling chamber (+4°C) and exposed to UV-light at the wave length of 366 nm for 24 h.

15 The polymer formed is ground up manually in a mortar and then dried in a vacuum-type desiccator for 3 h. The polymer is further ground in a mechanical mortar device (Retsch, Haan, Germany) for 20 min. The material is screened through a 25 μm screen. The remaining material is 20 ground and screened in two more turns. Small polymer particles from the screened material are removed by a sedimentation process in acetonitrile for 30 min in five turns.

The resulting material is packed in an HPLC steel 25 column (200 x 4.6 mm) in chloroform/acetonitrile (v/v, 3/17) at a pressure of 300 bars.

The column is arranged in an HPLC device (LKB, Bromma, Sweden) and washed therein with acetic acid/acetonitrile (v/v 1/9) at a flow rate of 1 ml/min for 1 h. 30 Subsequently, the column is equilibrated in acetonitrile/-acetic acid (v/v, 93/7) at a flow rate of 1 ml/min, a pressure of 30 bars and detection at 275 nm.

20 μg (R,S)-atenolol is injected in 20 μl of the eluant acetonitrile/acetic acid (v/v, 93/7).

35 $k'_R=1.18$ $k'_S=2.34$ $\alpha=2.0$ $R_S=0.5$

Example 4Preparation of a polymer selective for (S)(-)-metoprolol:

535 mg (2 mmol) (S)(-)-metoprolol is resolved in a 50 ml test tube in a mixture of 6 ml chloroform, 537 mg (6 mmol) methacrylate, 4.985 ml (24 mmol) ethylene glycol dimethacrylate and 56 mg (0.34 mmol) 2,2'-azobis(2-methyl propionitrile). The solution is cooled in an ice bath, and nitrogen gas is caused to flow through the solution for 20 min. The test tube is sealed. The tube is placed in a cooling chamber (+4°C) and exposed to UV-light at the wave length of 366 nm for 24 h.

The polymer formed is ground up manually in a mortar and then dried in a vacuum-type desiccator for 3 h. The polymer is further ground in a mechanical mortar device (Retsch, Haan, Germany) for 20 min. The material is screened through a 25 µm screen. The remaining material is ground and screened in two more turns. Small polymer particles from the screened material are removed in a sedimentation process in acetonitrile for 30 min in five turns.

The resulting material is packed in an HPLC steel column (200 x 4.6 mm) in chloroform/acetonitrile (v/v, 3/17) at a pressure of 300 bars.

The column is arranged in an HPLC device (LKB, Bromma, Sweden) and washed therein with acetic acid/acetonitrile (v/v, 1/9) at a flow rate of 1 ml/min for 1 h. Subsequently, the column is equilibrated in acetonitrile/acetic acid (v/v, 93/7) at a flow rate of 1 ml/min, a pressure of 30 bars and detection at 275 nm.

20 µg (R,S)-metoprolol is injected in 20 µl of the eluant acetonitrile/acetic acid (v/v, 93/7).

$k'_R=1.1$ $k'_S=3.1$ $\alpha=2.8$ $R_S=0.6$

Example 5Separation of enantiomers of timolol:

Two chiral solid-phase chromatography materials were prepared according to the Examples above, with methacrylic acid and itaconic acid, respectively, as monomers. Both

polymers with imprints of (S)(-)-timolol allowed base-line separation after application of a racemic mixture of timolol with R_S -values between 1.9 and 2.0 (see Table 1 and Figs 2A, B). The CSP obtained with methacrylic acid (MAA-CSP) also allowed separation of enantiomers of other β -blockers (see Fig. 1).

However, regarding the methacrylic acid polymer the resolution of racemic mixtures of metoprolol and atenolol was unsatisfactory (as shown), but propranolol was resolved in a satisfactory manner ($k'_R=1.0$; $\delta=2.8$; $R_S=1.3$). This agrees with the results of enantiomer separation of amino acid derivatives of structurally related molecules with MAA-polymers (9). Owing to the high optical rotation values of propranolol, the enantiomer separation thereof could also be determined polarimetrically when the test concentration was increased to 20 g/l. In this manner it was determined that the peaks obtained by separation of the enantiomers were identical with those as measured by means of UV-absorption.

Regarding itaconic acid based polymers with molecular imprints (ITA-CSP), not only were sharper peaks obtained, but a higher degree of selectivity was also exhibited. By using (S)(-)-timolol as print molecule and subsequently applying an artificial mixture of the racemic aryloxypropanolamines timolol, propranolol, atenolol and metoprolol (structures, see Fig. 1), (S)(-)-timolol was retained in the most efficient manner of them all, while the others were neither separated in their enantiomer forms nor particularly bonded to the polymer (propranolol, $k'_{rac}=0.2$; atenolol and metoprolol, $k'_{rac}=2.2$; timolol, $k'_R=2.5$ and $k'_S=3.6$; flow rate 0.4 ml/min, UV-detection at 275 nm).

These results are probably caused by the fact that the neighbouring carboxyl groups on the bifunctional monomer itaconic acid which is used have a more pronounced possibility of interactions (reciprocal actions) with the heterocyclic side chain of timolol.

Table 1

Chromatographic resolution of timolol on chiral solid phases prepared by the molecular imprinting method by using (S)(-)-timolol as print molecule

5	Functional monomer in the chiral solid phase	k'_R	α	R_S
	Methacrylic acid (MMA)	2.0	2.9	2.9
10	Itaconic acid (ITA)	1.4	2.5	1.9

Fig. 2 shows a diagram of the chromatographic resolution of timolol on polymers containing (A) methacrylic acid and (B) itaconic acid. The selected
15 optimised eluants were acetonitrile/acetic acid (93/7, v/v) for (A) and ethanol/tetrahydrofuran/acetic acid (50/40/10, v/v/v) for (B). The test volume was 20 μ l containing 20 μ g β -blockers, the flow rate was 1 ml/min and the pressure about 30 bars. All separations were
20 effected at ambient temperature and the UV-detection was made at 294 nm. The elution sequence was determined by injection of the pure enantiomers.

The capacity shown is perfectly well comparable with e.g. a biological alternative method using the protein
25 cellulase (6d). The quantity of timolol with optimal base-line separation was 18.9 and 19.9 μ g/g dry CSP, respectively, (Fig. 2) and in connection with the above-mentioned polarimetric study of propranolol with acceptable resolution but no base-line separation, 400 μ g/g dry CSP.
30

Literature

- (1) (a) Richards, R.; Tattersfield, A. E., Br. J. Clin. Pharmacol. 1987, 24, 485-491. (b) Kloosterman, M.; Elferink, V. H. M.; van Iersel, J.; Meijer, E. M.; Hulshof, L.; Beldon, R. A., Trends Biotechnol. 1988, 6, 251-256. (c) T.; Webb, J.; Bagwell, E.; Walle, K.; Daniell, H.; Gaffney, T., Biochem. Pharmacol. 1988, 37, 115-124. (d) Lindner, W.; Rath, M.; Stoschitzky, K.: Semmelrock, H. J., Chirality 1989, 1, 10-13.
- (2) (a) Katsuki, T., Tetrahedron Lett. 1984, 25 (26), 2821-2822. (b) Klunder, J. M.; Ko, S. Y.; Sharpless, K. B., J. Org. Chem. 1986, 51, 3710-3712.
- (3) (a) Matsuo, N.; Ohno, N., Tetrahedron Lett. 1985, 26 (45), 5533-5534. (b) Phillips, G. T.; Bertola, M. A.; Marx, A. F.; Koger, H. S., Eur. Pat. Appl. EP 256586, 1988, 9 pp.
- (4) Oy Star AB, Neth. Appl. NL 8500939, 1988, 27 pp.
- (5) (a) König, W. A.; Ernst, K., J. Chromatogr. 1983, 280, 135-141. (b) Isaksson, R.; Lamm, B. J., J. Chromatogr. 1986, 362, 436-438.
- (6) (a) Armstrong, D. W.; Ward, T. J.; Armstrong, R. D.; Beesley, T. E., Science 1986, 232, 1132-1135. (b) Okamoto, Y.; Aburatami, R.; Hatano, K.; Hatada, K., J. Liq. Chromatogr. 1988, 11 (9-10), 2147-2163. (c) Aboul-Enein; Hassan, Y.; Islam, M. R., J. Chromatogr. 1990, 511, 109-114. (d) Erlandsson, P.; Marle, I.; Hansson, L.; Isaksson, R.; Pettersson, G.; Pettersson, C., J. Am. Chem. Soc. 1990, 112, 4573-4574.
- (7) (a) Review: Wulff, G. In Polymeric Reagents and Catalysts; Ford, W. T., Ed.: ACS Symposium Series 208;

11

American Chemical Society: Washington DC, 1986; pp
186-230. (b) Sellergren, B.; Lepistö, M.; Mosbach, K., J.
Am. Chem. Soc. 1988, 110, 5853-5860. (c) Shea, K. J.;
Sasaki, D. Y., J. Am. Chem. Soc. 1989, 111, 3442-3444, (d)
5 Ekberg, B.; Mosbach, K., Trends Biotechnol. 1989, 7,
92-96.

(8) Tate B., Adv. Polym. Sci. 1969, 5, 214-232.

10 (9) Andersson, L. I., Doctoral Thesis, University of Lund,
Sweden, 1991.

15

20

25

30

35

CLAIMS

1. Method for separating enantiomers of a derivative
5 of an aryloxiopropanolamine, characterised in
that the derivative is contacted with a chiral solid-phase
chromatography material containing molecular imprints of
an optically pure enantiomer of the derivative to be
separated.

10 2. The method as claimed in claim 1, characterised
in that, as chiral solid-phase chromatography material,
use is made of a polymer which is prepared by polymerisation
of a monomer in the presence of a cross-linking agent and,
15 as print molecule, use is made of the optically pure enantiomer
of the derivative to be separated.

3. Chiral solid-phase chromatography material for use
in the separation of enantiomers of derivatives of aryl-
oxipropanolamine, characterised in that it
20 consists of a polymer prepared by polymerisation of a
monomer in the presence of a cross-linking agent and an
optically pure enantiomer of the derivative to be separated,
a molecular imprint of the optically pure enantiomer
being formed in the polymer by non-covalent interactions
25 between the monomer and the optically pure enantiomer.

4. The chiral solid-phase chromatography material as
claimed in claim 3, characterised in that
the derivative of aryloxiopropanolamine, which is used in
the formation of the molecular imprint, is timolol,
30 propranolol, metoprolol or atenolol.

5. The chiral solid-phase chromatography material as
claimed in claim 3 or 4, characterised in
that the monomer is a carboxyl-functional monomer.

6. The chiral solid-phase chromatography material as
35 claimed in claim 5, characterised in that
the carboxyl-functional monomer is itaconic acid.

13

7. The chiral solid-phase chromatography material as claimed in claim 5, characterised in that the carboxyl-functional monomer is methacrylic acid.

8. The chiral solid-phase chromatography material as claimed in any one of claims 3-7, characterised in that the cross-linking agent is ethylene glycol dimethylacrylate.

9. Use of the molecular imprinting method for preparing a chiral solid-phase chromatography material for use in the separation of enantiomers of a derivative of an aryloxipropanolamine.

15

20

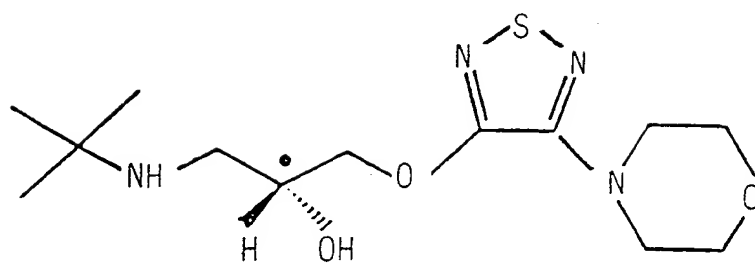
25

30

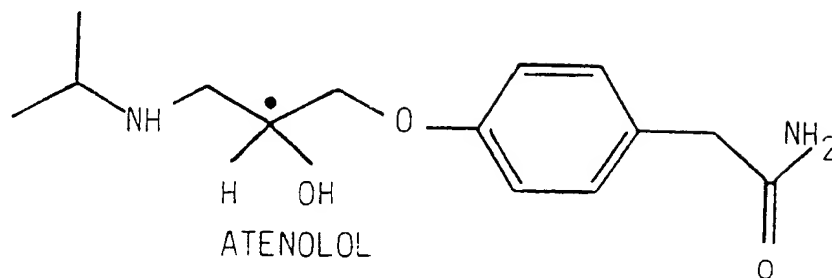
35

1/2

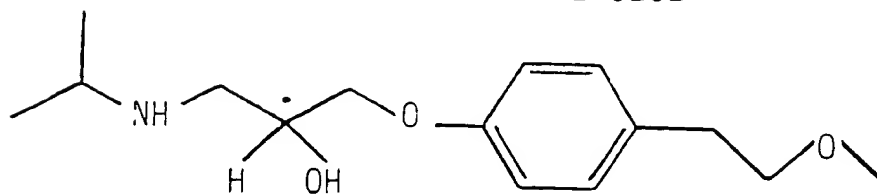
FIG.1



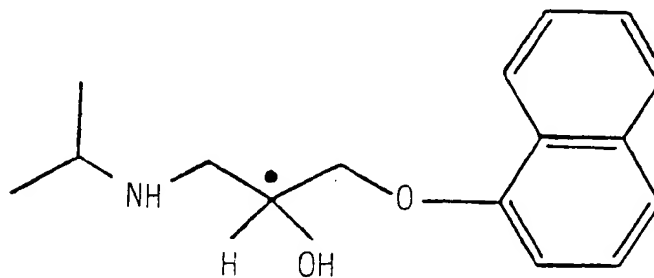
(S)-(-)-TIMOLOL
USED AS PRINT MOLECULE



ATENOLOL



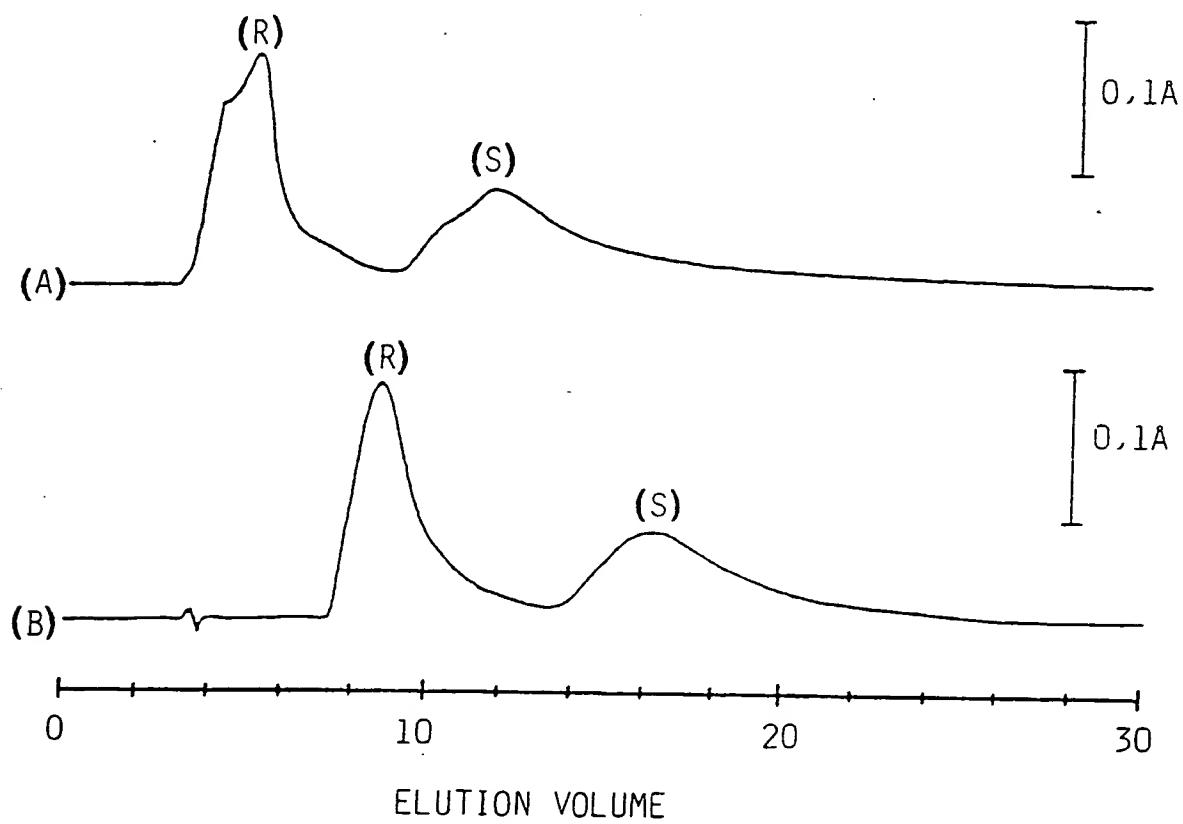
METOPROLOL



PROPRANOLOL

2/2

FIG. 2



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 92/00751

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 B 57/00, C 07 C 213/10, 231/20, C 07 D 417/04														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%; border-bottom: 1px solid black;">Classification System</td> <td style="border-bottom: 1px solid black;">Classification Symbols</td> </tr> <tr> <td style="height: 40px; vertical-align: bottom;">IPC5</td> <td style="vertical-align: bottom;">C 07 B; C 07 C; C 07 D</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div> <p style="margin-top: 10px;">SE,DK,FI,NO classes as above</p>			Classification System	Classification Symbols	IPC5	C 07 B; C 07 C; C 07 D								
Classification System	Classification Symbols													
IPC5	C 07 B; C 07 C; C 07 D													
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category *</th> <th style="width: 70%;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 20%;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top;">P,X</td> <td>J. Am. Chem. Soc., Vol. 113, 1991 Lutz Fischer et al.: "Direct Enantioseparation of Beta-Adrenergic Blockers Using a Chiral Stationary Phase Prepared by Molecular Imprinting", see page 9358 - page 9360 --</td> <td style="vertical-align: top;">1-9</td> </tr> <tr> <td style="vertical-align: top;">X</td> <td>Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution on molecularly imprinted polymers prepared with only non-covalent and non-ionic interactions", see page 313 - page 322 --</td> <td style="vertical-align: top;">1-9</td> </tr> <tr> <td style="vertical-align: top;">A</td> <td>Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution of amino acid derivatives on molecularly imprinted polymers as monitored by potentiometric measurements", see page 323 - page 331 --</td> <td style="vertical-align: top;">1-9</td> </tr> </tbody> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	P,X	J. Am. Chem. Soc., Vol. 113, 1991 Lutz Fischer et al.: "Direct Enantioseparation of Beta-Adrenergic Blockers Using a Chiral Stationary Phase Prepared by Molecular Imprinting", see page 9358 - page 9360 --	1-9	X	Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution on molecularly imprinted polymers prepared with only non-covalent and non-ionic interactions", see page 313 - page 322 --	1-9	A	Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution of amino acid derivatives on molecularly imprinted polymers as monitored by potentiometric measurements", see page 323 - page 331 --	1-9
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³												
P,X	J. Am. Chem. Soc., Vol. 113, 1991 Lutz Fischer et al.: "Direct Enantioseparation of Beta-Adrenergic Blockers Using a Chiral Stationary Phase Prepared by Molecular Imprinting", see page 9358 - page 9360 --	1-9												
X	Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution on molecularly imprinted polymers prepared with only non-covalent and non-ionic interactions", see page 313 - page 322 --	1-9												
A	Journal of Chromatography, Vol. 516, 1990 Lars I. Andersson et al.: "Enantiomeric resolution of amino acid derivatives on molecularly imprinted polymers as monitored by potentiometric measurements", see page 323 - page 331 --	1-9												
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="height: 40px; vertical-align: bottom;">10th February 1993</td> <td style="vertical-align: bottom;">15 -02- 1993</td> </tr> <tr> <td style="border-bottom: 1px solid black;">International Searching Authority</td> <td style="border-bottom: 1px solid black;">Signature of Authorized Officer</td> </tr> <tr> <td style="text-align: center; vertical-align: bottom;">SWEDISH PATENT OFFICE</td> <td style="vertical-align: bottom;">Eva Johansson</td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	10th February 1993	15 -02- 1993	International Searching Authority	Signature of Authorized Officer	SWEDISH PATENT OFFICE	Eva Johansson				
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report													
10th February 1993	15 -02- 1993													
International Searching Authority	Signature of Authorized Officer													
SWEDISH PATENT OFFICE	Eva Johansson													

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Journal of Chromatography, Vol. 470, 1989 Daniel J. O'Shannessy et al: "Recent advances in the preparation and use of molecularly imprinted polymers for enantiomeric resolution of amino acid derivatives", see page 391 - page 399 --	1-9
A	J. Am. Chem. Soc., Vol. 110, 1988 Börje Söllergren et al: "Highly Enantioselective and Substrate-Selective Polymers Obtained by Molecular Imprinting Utilizing Noncovalent Interactions. NMR and Chromatographic Studies on the Nature of Recognition", see page 5853 - page 5860 --	1-9
A	Journal of Liquid Chromatography, Vol. 13, No. 15, 1990 Günter Wulff et al: "Template imprinted polymers for HPLC separation of racemates", see page 2987 - page 3000 --	1-9
A	J. Mol. Recognit., Vol. 2, No. 1, 1989 Daniel J. O'Shannessy et al: "Molecular recognition in synthetic polymers. Enantiomeric resolution of amide derivatives of amino acids on molecularly imprinted polymers", see page 1 - page 5 -- -----	1-9

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 92/00751

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 08/01/93
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date